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Full Length Research Paper

Identification of bio-active components in leaf extracts of Aloe vera, Ocimum tenuiflorum (Tulasi) and Tinospora cordifolia (Amrutballi)

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Plants are an integral part of life in many indigenous communities. Besides, being the source of food, fodder, fuel, etc., the use of plants as herbal medicines in curing several ailments goes parallel with the human civilization. The present study was carried out to identify the bio-active compounds in various leaf extracts of Aloe vera, Ocimum tenuiflorum and Tinospora cordifolia using different phytochemical screening tests. Total phenolic content (TPC) was determined by Folin-Ciocalteu reagent assay method and total flavonoid content (TFC) using aluminium chloride colorimetric method in the sample extracts. The phytochemical screening results revealed that all the extracts of A. vera, O. tenuiflorum and T. cordifolia are positive for alkaloids, but flavonoids and tannins are present only in some of the extracts. Positive results for saponins and terpenoids are obtained only for T. cordifolia extracts. TPC of A. vera was high in ethanolic extract (138.13 mg/g) compared to the other two solvents, whereas methanolic extract of O. tenuiflorum (114.34 mg/g) and aqueous extract (465.82 mg/g) of T. cordifolia exhibited maximum TPC comaparitively. TFC of A. vera was higher in methanol extract (88.59 mg/g) compared to the other two solvents, whereas methanol (96.34 mg/g) and ethanol (95.46 mg/g) extracts exhibited similar TFC for O. tenuiflorum compared to aqueous extract and for T. cordifolia ethanol extract (208.36 mg/g), exhibited maximum TFC comaparitively. The results of all the three sources were found to be highly significant. However, there is a need to exploit natural sources with medicinal value not only to be used in medical field but also for developing healthy clothing.

Key words: Phytochemical, Aloe vera, Ocimum tenuiflorum, Tinospora cordifolia.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially

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useful new compounds for the development of chemotherapeutic agents. Besides, being the source of food, fodder, fuel, etc., the use of plants as herbal medicines in curing several ailments goes parallel to the human civilization. India is endowed with a rich wealth of medicinal plants, being perhaps the largest producer and rightly acclaimed as the botanical garden of the world (Dubey et al., 2004). Natural products are the source of synthetic and traditional herbal medicine. According to the world health organization (WHO), about 80% of the world’s people depend on traditional indigenous medicines, since a large majority of rural people in the developing countries still use these medicines as the first defense in health care (Goleniowski et al., 2006). In India, knowledge about medicinal properties of plants is the basis for their uses as home remedies; plants serve as source of many traditional medicines and continue to provide new remedies to mankind. Indigenous use of medicinal plants all over the world precedes the origin of modern medicine in healthcare system (Aburjai et al., 2007). Certain plant drugs used in modern medicine have ethno-botanical background (Dev, 1997; Fabricant and Farnsworth, 2001). Crude plant extracts (e.g. infusion, tincture, decoction or others) are traditionally used by populations all over the world for medicinal purposes. Although their effectiveness and mechanisms of action have not been scientifically tested in majority of the cases, they often mediate beneficial responses due to their bioactive chemical components (Barnes et al., 2007).

Phytochemical is a natural bioactive compound found in different parts of plants, fruit, flower, stem, leaf and root. Phenolic compounds, cyclic derivatives of benzene with one or more hydroxyl groups associated with the aromatic ring, account for one of the largest and most widely distributed group of phytochemicals (Andjelkovic et al., 2006). They vary considerably in structure with over 8000 naturally-occurring compounds having been identified (Balasundram et al., 2005). These bioactive compounds work with nutrients and fibers to act as defense system against diseases. Metabolites comprise of alkaloids, terpenoids and phenolic compounds (Krishnaiah et al., 2007) and many more such as flavonoids, tannins, etc.

_Aloe vera_ is a semi tropical plant which belongs to the family Liliaceae, and is used by herbalists for the treatment of different human disorders. _A. vera_ contains amino acids, lipids, sterols, tannins and flavonoids. Therefore, this plant is found useful in the treatment of wound, burns, skin disorders and anti-inflammatory activity. A mucilaginous gel from the parenchymatous tissue of the leaf has been used for topical treatment of skin burns and wounds (Rovatti and Brennan, 1959). Also often mentioned are the antibacterial, antifungal and even antiviral properties demonstrated by the leaf gel (Klein and Penneys, 1988; Marshall, 1990; Ahmad et al., 1993). As regards to the healing properties, many researches have demonstrated that the mucilaginous polysaccharides contained in the clear pulp of _A. vera_ leaf are the major ingredient responsible for the healing. However, new evidence has shown that emodin, one of the derivatives of anthraquinones produced by superficial pericyclic cells, is also capable of promoting the repair of rats’ excisional wounds via stimulating tissue regeneration (Eshun and He, 2004; Tang et al., 2007).

_Ocimum tenuiflorum_, also called as _Ocimum sanctum_ is considered a sacred plant/herb in India, known as “Tulsi” or “Tulasi” in Hindi or Holy Basil in English. _Tulasi_ belongs to the family Lamiaceae (Labiatae). The plant has yielded different types of constituents including phenol derivatives, flavonoids, phenyl propanoids, triterpenoids (Manitto et al., 1974; Siddiqui et al., 2007a), steroids and steroidal glycosides (Siddiqui et al., 2007b). Wagner et al. (1994) mentioned the use of Basil leaves for a variety of conditions such as catarhal bronchitis, bronchial asthma, dysentery, dyspepsia, skin diseases, chronic fever, hemorrhage, helminthiasis and topically for ring worms (Singh et al., 1980; Warier, 1995; Kirtikar and Basu, 1993). The oil has antibacterial and antifungal properties (Manitto et al., 1974) and thymol, a constituent of _O. sanctum_ is well known as an antiseptic agent (Dikshit and Husain, 1984).

_Tinospora cordifolia_, known as _Amrutballi_ is, a climber plant which belongs to the family Menispermaceae. Extracts of this plant has been shown to possess many therapeutic properties including general tonic, anti-inflammatory, anti-arithmetic, anti-malarial, aphrodisiac (Rao et al., 2008), anti-allergic (Nayampalli et al., 1986), anti-diabetic (Wadood et al., 1992), anti-hepatotoxic (Rege et al., 1984) and antipyretic (Kumar and Shrivastav, 1995). The phytochemical constituents in the individual extract have not been specified, it may be possible that multiple constituents of _T. cordifolia_ exhibit similar pharmacological properties irrespective of their nature. Although, the active components responsible for therapeutic effects of _T. cordifolia_ are not well defined; phenylpropanoid glycosides such as cordifolioside A, cordifolioside B and syringin, have been reported to be main immunomodulatory active components (Maurya et al., 1996; Kapil and Sharma, 1997; Cho et al., 2001).

Natural medicinal plants abundantly available worldwide, are now getting more attention as they possess numerous benefits to society or indeed to all mankind, especially in the field of medicine and pharmacological. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. The organic compounds usually related with physiological actions on the human body include alkaloids, phenolics, flavonoids, tannins, terpenoids, and steroids (Yadav and Agarwala, 2011). Therefore, the aim of present study was undertaken to screen various bio-active compounds present in the leaf extracts of _Aloe vera_, _O. tenuiflorum_ and _T. cordifolia_ and
to evaluate the total phenolic content and total flavonoid content in the plant sources.

MATERIALS AND METHODS

Plant sources

Fresh leaves of *A. vera*, *O. tenuiflorum* and *T. cordifolia* were selected for the study from the University of Agricultural Sciences, Dharwad campus.

Chemicals and reagents

Ethyl alcohol, methanol, chloroform, ammonia, aluminium chloride, hydrochloric acid, sulphuric acid, ferric chloride, sodium hydroxide, sodium chloride, sodium carbonate, sodium nitrite, lead acetate, and gelatin were purchased from Rankem chemicals, Bangalore which were of analytical grade. Folin-Ciocalteu and Dragendorff’s reagent was purchased from Merck, Germany. Gallic acid and rutin standards were purchased from Sigma-Aldrich Co. (St.Louis, USA).

Preparation of extracts

The selected plant leaves were cleaned with distilled water and shade dried for a 2 h at room temperature (25 ±2°C) to remove the traces of moisture. Two grams of fresh leaf of each sample were weighed, chopped into fine pieces and ground in a pestle and mortar. The ground samples were mixed with 25 ml of each solvent, namely, ethanol, methanol, chloroform and distilled water separately. Incubated under agitation at 200 forward and backward strokes in incubator shaker (Inkarp, Germany) for 24 h at room temperature 25°C. The extracts were centrifuged at 5000 rpm at room temperature (C24 Plus, RemiElektrotechnik, Mumbai) and the supernatants were separated. Residue was re-extracted with 25 ml of the respective solvent and the process was repeated one more time. The supernatants obtained were pooled and the extracts obtained were measured, and filtered using Whatman filter paper No. 40 (125 mm); extracts were stored at 8°C until further analysis within a week.

Phytochemical analysis

The chemical tests described by Ajayi et al. (2011), Raaman (2006) and Rahul et al. (2010) were adopted for the screening of various phytoconstituents like alkaloids, flavonoids, tannins, saponins and terpenoids in the extracts of *A. vera*, *O. tenuiflorum* and *T. cordifolia*.

Test for tannins and phenolic compounds

**Ferric chloride test:** 1 ml of the extract was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5% FeCl₃ was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of tannins.

**Gelatin test:** 2 ml of 1% solution of gelatin containing 1% NaCl is added to 1 ml of the extract. White precipitate indicates the presence of phenolic compounds.

**Lead acetate test:** 3 ml of 10% lead acetate solution was added to 1 ml of the extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds.

Test for flavonoids

**Ammonia test:** A few drops of 1% NH₄ solution was added to 1 ml of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

**Sodium hydroxide test:** Few drops of 20% NaOH solution was added to 1 ml of the extract. On addition of HCl, the changed yellow colour of the extract turns to a colourless solution that depicted the presence of flavonoids.

Test for alkaloids

**Dragendorff test:** To 1 ml of the extract, few drops of Dragendorff’s reagent were added. A prominent yellow precipitate indicates the positive test.

**Wagner test:** Few drops of Wagner’s reagent were added by the side of the test tube to 1 ml of extract. A reddish-brown precipitate confirms the test as positive.

**Mayer test:** 1 ml of the extract was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with a few drops of Mayer’s reagent. The turbidity of the extract filtrate on addition of Mayer’s reagent was taken as evidence of the presence of alkaloids in the extract.

Test for saponins

**Foam test:** About 1 ml of the sample extract was boiled in 20 ml of distilled water in a water bath and filtered; 10 ml of the filtrate was mixed with the 5 ml of distilled water and mixed vigorously for 15 min to form a stable persistent froth. The presence of froth after 5 min was taken as an indication of presence of saponins.

Test for terpenoids

**Salkowski test:** 1 ml of each extract was mixed with 0.5 ml of chloroform and 1 ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish brown colouration of the interface formed to show positive results for the presence of terpenoids.

Total phenolic content (TPC)

TPC in the extracts was determined by Folin-Ciocalteu assay method (Singleton and Rossi, 1965) with little modification using gallic acid as the reference standard. Briefly, all the solvent extracts were diluted to appropriate volumes and were mixed with 2 ml of 10% Na₂CO₃ solution. Incubated at room temperature for 3 min, 100 µl of Folin-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 min at room temperature under dark, the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer (Varian, Middelburg, Netherlands). The TPC was expressed as gallic acid equivalent (GAE) in milligrams per gram of fresh leaf (Figure 2).

Total flavonoid content (TFC)

TFC was determined by aluminium chloride colorimetric method (Yun et al., 2009; Jyoti et al., 2014) with minor modification. Aliquots (1 ml) of appropriately diluted extracts or standard solutions were pipette into 15 ml polypropylene conical tubes
Table 1. Phytochemical screening of various extracts from selected plant sources.

<table>
<thead>
<tr>
<th>Chemical screening tests</th>
<th>Aloe vera (Solvent extracts)</th>
<th>O. tenuiflorum (Solvent extracts)</th>
<th>T. cordifolia (Solvent extracts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E*</td>
<td>M*</td>
<td>C*</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dragendorff’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wagner’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sodium hydroxide test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salkowski test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

E*: Ethanol, M*: methanol, C*: chloroform, A*: aqueous, (+) = positive; (-) = negative.

containing 2 ml double distilled H$_2$O and mixed with 0.15 ml of 5% NaNO$_2$. After 5 min, 0.15 ml of 10% AlCl$_3$.6H$_2$O solution was added and the mixture was allowed to stand for another 5 min, and then 1 ml of 1 M NaOH was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at $\lambda_{415}$ nm using the UV-Visible Spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). TFC was calculated using the standard rutin curve and expressed as mg rutin equivalent (mg RE) per gram of fresh leaf (Figure 3).

RESULTS AND DISCUSSION

Yield of extracts

The yield of Aloe vera using ethanol and methanol solvents (45 ml of extract/50 ml of solvent) was found to be higher than aqueous (43 ml of extract/50 ml of solvent). O. tenuiflorum exhibited higher yield in methanol (41 ml of extract/50 ml of solvent) followed by ethanol (40 ml of extract/50 ml of solvent) and aqueous (39 ml of extract/50 ml of solvent) extracts (Figure 1). On the other hand, aqueous (36 ml of extract/50 ml of solvent) extract of T. cordifolia was found to be maximum as compared to methanol (35 ml of extract/50 ml of solvent) and ethanol (32 ml of extract/50 ml of solvent) extracts.

Phytochemical screening

Table 1 records the phytochemical screening of various extracts of selected species. It is observed from the Table that all the extracts of A. vera, O. tenuiflorum and T. cordifolia exhibited positive results for alkaloids proved by Dragendorff’s and Wagner’s tests.

The presence of flavonoids was positively proved by both tests (ammonia and sodium hydroxide) in ethanol and methanol extracts of A. vera, methanol and aqueous extracts of tulasi and ethanol, methanol and aqueous extracts of tinospora.

All the extracts of A. vera and O. tenuiflorum depicted the presence of tannins as proved by lead acetate test whereas only ethanol, methanol and aqueous extracts of T. cordifolia showed positive results with lead acetate test. However, ethanol, methanol, chloroform extracts of O. tenuiflorum and methanol and aqueous extracts of T. cordifolia exhibited positive results while chloroform and aqueous extracts of O. tenuiflorum and methanol, chloroform and aqueous extracts of T. cordifolia depicted the presence of tannins using ferric chloride and gelatin tests, respectively.

None of the extracts of A. vera and O. tenuiflorum proved the presence of saponins except chloroform extract of T. cordifolia using foam test. Similarly, only ethanol, methanol and chloroform extracts of T. cordifolia proved the presence of terpenoids with Salkowski test.

Total phenolic content (TPC)

TPC of selected plant sources is described as shown in
Table 2. Total phenolic content (TPC) of the plant leaf extracts.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Total phenolic content (GAE* mg/g)</th>
<th>Aloe vera</th>
<th>O. tenuiflorum</th>
<th>T. cordifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>94.42±4.92</td>
<td>80.82±8.63</td>
<td>465.82±23.04</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>138.13±6.63</td>
<td>113.07±9.81</td>
<td>264.06±18.41</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>95.20±3.23</td>
<td>114.34±11.86</td>
<td>301.42±29.69</td>
<td></td>
</tr>
</tbody>
</table>

GAE*: Gallic acid equivalent.

Table 3. Total flavonoid content (TFC) of the plant leaf extracts.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Total phenolic content (RE* mg/g)</th>
<th>Aloe vera</th>
<th>O. tenuiflorum</th>
<th>T. cordifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>72.28±8.70</td>
<td>80.82±7.25</td>
<td>465.82±6.61</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>138.13±8.57</td>
<td>113.07±4.12</td>
<td>264.06±2.86</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>95.20±8.38</td>
<td>114.34±5.85</td>
<td>301.42±7.59</td>
<td></td>
</tr>
</tbody>
</table>

RE*: Rutin equivalent.

Table 2. The results revealed that Aloe vera leaves contained higher TPC in ethanol (138.13 mg/g) extract followed by methanol (95.20 mg/g) and aqueous (94.42 mg/g) extracts. On the other hand, methanol (114.34 mg/g) extract of O. tenuiflorum yielded higher amount of TPC compared to ethanol (113.07 mg/g) and aqueous (80.82 mg/g) extracts. Further, T. cordifolia exhibited maximum TPC using aqueous (465.82 mg/g) against the phenolic concentration of methanol (301.42 mg/g) and ethanol (264.06 mg/g) solvents.

**Total flavonoid content (TFC)**

TFC of the plant leaf extracts is described as shown in Table 3. The results revealed that in A. vera leaf extracts...
higher TFC was obtained in methanol (88.59 mg/g) followed by ethanol (76.50 mg/g) and aqueous (72.28 mg/g) extracts. Whereas, for O. tenuiflorum methanol (96.34 mg/g) and ethanol (95.46 mg/g) extracts exhibited similar TFC with not much significant difference and for aqueous extract it was 61.84 mg/g. For T. cordifolia, TFC was higher in ethanol extract (208.36 mg/g) followed by aqueous (178.43 mg/g) and methanol (132.59 mg/g) extracts. However, the treatments of all three sources were found to be highly significant with respect to solvents which imply that there is greater influence of solvents on extraction of various phytoconstituents.

**Conclusion**

India has a rich flora used in traditional medical treatments; the medicinal properties of these plants could be based on the therapeutic and antioxidant effects of different phytochemicals present in them. The results revealed that alkaloids were found to be present in all the extracts of A. vera, O. tenuiflorum and T. cordifolia. However, flavonoids were present in ethanol, methanol and aqueous extracts of A. vera and T. cordifolia, and methanol, chloroform and aqueous extracts of O. tenuiflorum. Positive results for saponins and terpenoids
were obtained only with \textit{T. cordifolia}. Whereas when compared in between the samples, TPC was high in ethanol extract of \textit{Aloe vera}, methanol extract of \textit{O. tenuiflorum} and aqueous extract of \textit{T. cordifolia}. TFC was high in methanol extract of \textit{A. vera}, methanol and ethanol extracts of \textit{O. tenuiflorum} and ethanol extract of \textit{T. cordifolia}. Furthermore, the results of all the three sources were found to be highly significant. Hence, there is need to explore the applicability of these plant resources which are rich in phytochemicals/phenolics and may have beneficial effects on health.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES


Serjania erecta Radlk. improves endothelial function in isolated aortic rings

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Arterial hypertension presents a high incidence in the population, and it is a chronic degenerative disease. The roots of Serjania erecta Radlk. popularly known as “cipó-cinco-folhas”, are used as a decoction against hypertension. This plant belongs to the Sapindaceae family and is plentiful in the Brazilian swamps. The present study was designed to study the effects of daily intake of S. erecta root decoction on mean arterial pressure (MAP), heart rate (HR) and vascular reactivity in thoracic aorta rings in spontaneous hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). The decoction was prepared from the powder of the roots at a final concentration of 5% in solution, which is an appropriate level of administration according to some modifications of the dessert test, for 32 consecutive days. The MAP and HR were measured directly and presented no changes between groups. Vascular reactivity was tested by concentration-effect curves for noradrenaline obtained from thoracic aorta rings with (+E) and without (-E) endothelium. In SHR, treatment reduced vascular reactivity (+E) (0.92 ± 0.18 vs. control SHR: 1.68 ± 0.26 g), and the presence of L-NAME abolished this effect (treated SHR: 1.94 ± 0.30 g). The aorta rings (-E) exhibited no changes. In conclusion, the decoction of S. erecta roots is effective in increasing endothelial function in SHR rats. However, under these conditions, the decoction did not change the MAP and HR. Therefore, this part of the plant promotes nitric oxide (NO) production by the endothelium, which may promote blood flow to the tissues; thus, its use is justified as adjunct for the treatment of hypertension.

Key words: Serjania erecta Radlk., hypertension, endothelium function, nitric oxide.

INTRODUCTION

Hypertension is considered a chronic degenerative disease; it presents no exacerbated symptoms and has serious consequences if not treated, targeting organs such as heart, kidney, brain and eyes. High...
pressure is considered one of the most common causes of morbidity in adults (Nobre, 2010; Ripley et al., 2015). A major problem in the treatment of hypertension is poor patient adherence. It has been estimated that 30 to 50% of patients discontinue therapy or take only one of the medicines prescribed within the first year of the treatment (Carvalho et al., 1991; Ross et al., 2004; Wilson et al., 2005). Generally, hypertensive elderly patients report that the need of medication is only associated with the presence of symptoms (Carvalho et al., 1998). Patients still hold beliefs considering the benefits and side effects of a prescribed medicine (Horne and Weinman, 1999; Magadza et al., 2009). Some elderly report dislike of allopathic medicines, believing that their chemicals are very bad for the body, producing many side effects (Carvalho et al., 1998). In the countryside of Sao Paulo State, Brazil, 15% of hypertensive patients reported the use of home medications for the treatment of hypertension, and 45% of these patients reported the use of teas (Boldo, lemongrass, rosemary, garlic water) as supporting treatments, especially when they realize their arterial pressure is elevated (Péres et al., 2003). In this sense, validating plants as potential hypertension treatments might be an improvement by promoting better compliance for hypertension treatment.

The use of plants for primary healthcare in underdeveloped and developing countries ranges from 65 to 80%, according to the World Health Organization. This is mainly due to poverty and lack of access to synthetic drugs (Akerele, 1993). The Mato Grosso do Sul State, with savannah and wetland, typical Brazilian biomes, has great potential to yield compounds with pharmacological activities (Proença et al., 2000). One of the species that requires attention is *Serjania erecta* Radlk., Sapindaceae family, known as “limbó-bravo”, barkcloth, vine-of-limbó, vine-five plants or five leaves. This plant is an erect shrub, half climbing, 1 to 3 m tall, and blooms in January (Pott and Pott, 1994). The fruits of this genus are edible (Pott et al., 2004), and the oil from its seeds contains fatty acids, with a great predominance of eicosanoic acid (Mayworm and Salatino, 1996). Ethnobotanical studies, reflecting the beliefs and cultural aspects of the population, indicated that the tea from the leaves of *S. erecta* acts against gastric ulcers and the decoction of the roots acts against hypertension (Guarim, 1996, 2000).

Pharmacological evaluation of hydroalcoholic extracts of the stem and leaves of *S. erecta* showed topical anti-inflammatory activity in mice (Gomig et al., 2008). Methanol and chloroform extracts of the stem and leaves of *S. erecta* have gastroprotective action, showing no acute toxicity (Castelo et al., 2009). However, the possible effect of the roots of this plant against hypertension has not been tested via controlled studies in laboratory animals. Therefore, the aim of this study was to explore the chronic administration of the decoction of the *S. erecta* roots on the mean arterial pressure and heart rate of spontaneous hypertensive rats (SHR) and Wistar-Kyoto rats (WKY, control for SHR), as well as the vascular reactivity of these animals. In addition, this study also evaluated these animals’ exploratory behaviour in the open field and anxiety in the elevated plus maze.

**METHODOLOGY**

**Animals**

Spontaneously hypertensive rats (SHR, ~200 g initial) and their control, Wistar-Kyoto rats (WKY, ~200 g initial), acquired from the CEMIB - Multidisciplinary Center for Biological Research in the Area of Science in Laboratory Animals, UNICAMP, were used. The rats were kept at a controlled temperature (23 ± 2°C) and subjected to a light:dark cycle of 12:12 h. They were kept in individual cages with free access to tap water and food (Biobase®). T experiments took place between 8:00 and 16:00 h. The procedures were approved by the Ethics Committee in Animal Experimentation of the Institute of Biosciences of Botucatu, UNESP (32/08-CEUA).

**Plant collection**

The roots of *S. erecta* were collected in the city of Aquidauana, MS, Brazil, in September 2008; they were identified by A. Pott and deposited in the Herbarium of Embrapa Beef Cattle, Campo Grande, MS. The roots were washed and dried in the shade. They were crushed in a knife mill at the Institute of Chemistry of the Federal University of Mato Grosso do Sul (UFMS), Campo Grande-MS, to produce a coarse powder.

For phytochemistry analysis, standardized tests were used to assess which secondary metabolites were present in the roots using methodology described by Mattos (1997). In the literature, there are few phytochemical studies on this species, especially for the roots. The phytochemistry concerning the decoction is still missing.

**Decoction preparation and administration**

The decoction was prepared according to the 2nd edition of the Pharmacopoeia of the United States of Brazil (1959), which yields a final concentration of 5% plant extract. The decoction was administered using a modified dessert test according to Johnson and Schwob (1975). The original dessert test consists of offering a condensed milk solution at 30%, which is highly palatable and quickly ingested by rats. In the present study, the decoction was prepared in the morning with a volume of water that was 15% less than that required for the final concentration. After cooling, the remaining 15% volume was made up with condensed milk, which also produces a palatable solution. Thus, the final suspension of *S. erecta* roots was adjusted to 5% plant extract.

Each animal was offered 10 ml of this solution in graduated burettes placed in front of the cages at approximately 09:00. The solution was successfully ingested in a maximum time of 15 min for the rats. The percentage of treatments successfully administered was 100%. On rare occasions (twice), a rat took 45 min to drink 10 ml of the sweetened solution. Thus, the rats ingested equal amounts of decoction and the entire portion of the 5% of the plant in a relatively short period of time. In this way, the solution was not exposed to light for a long time, limiting potential changes in its active ingredients.

Half of the rats of each strain received a decoction dissolved in
the condensed milk, and the other half received the same volume of a 15% condensed milk solution. The treatment was performed over a period of 32 consecutive days. The animals had free access to water and food at all times, and were free to choose whether to ingest the solution or not. In order to ensure that the daily condensed milk intake did not alter the blood glucose of the animals after treatment, a small sample of blood was collected prior to euthanasia (approximately 2 h after administration of the decoction) and before collection of thoracic aortic rings. Blood glucose was analysed using glucose dosage strips (Prestige Smart System®). In this study, gavage procedure was avoided to minimize any further stress in the SHR, which would increase the blood pressure, especially for a chronic treatment.

Drugs

The drugs used included norepinephrine bitartrate, acetylcholine chloride, sodium nitroprusside and methyl ester of N-nitro-L-arginine (L-NAME; all purchased from Sigma Chemical Co., St. Louis, Missouri, USA). All drugs were dissolved in Krebs-Henseleit solution prepared immediately before use.

Exploratory and anxiety tests: Open field (OF) and elevated plus maze (EPM)

The treated SHR presented an apparent docility. Another point to consider in hypertension is its association with anxiety states. Increase in anxiety enhances the risk of hypertension (Ginty et al., 2013; Garcez et al., 2014). Thus, the possibility that the decoction might act centrally as an anxiolytic was tested. Following 30 days of treatment, the rats were exposed to tests in the OF and EPM. On the morning of the test, at approximately 8:00 h, the animals received the decoction (or control) as usual and were transferred to the test room for adaptation.

The tests were conducted between 13:00 and 16:00 h. The animals were exposed for 3 min in the OF, and parameters such as rearing and exploratory activity and the number of squares that were crossed were analysed. Then, the animals were placed in the EPM, and spatiotemporal measures of avoidance of open arms (time spent in the arms) and total number of entries into the arms of the apparatus were evaluated in order to assess anxiety (Pellow et al., 1985).

Direct measurement of blood pressure and heart rate

Mean arterial pressure (MAP) and heart rate (HR) were recorded in non-anesthetized rats with free movement. A day before the recordings, the animals were anesthetized with ketamine (80 mg/kg, Dopalen®, Vetbrands) plus xylazine (20 mg/kg, Anasedan®, Vetbrands). Then, they underwent femoral artery cannulation with a PE 10 polyethylene tube (0.024" OD × 0.011" ID), which was connected to a PE 50 polyethylene tubing (0.038" Od × 0.023" ID). The catheter was conducted subcutaneously and was externalized by the animals’ neck, allowing free movement.

On the next day, the PE 50 tube was connected to a pressure transducer (TSD104A, Biopac Systems) coupled to a multichannel recorder (Model M100A-CE, IC Biopac Systems, Santa Barbara, California), which was connected to computer for data acquisition. A solution of heparin (10 UI/ml) in a volume of 0.2 ml was used to flush the catheters only once in a previous connection to the transducer to facilitate the blood flow and avoid clot formation in the catheter. The recording was made for approximately 40 min.

Assessment of vascular reactivity test in vitro

After the blood pressure measurements, the animals were anesthetized with pentobarbital sodium, 50 mg/kg intraperitoneally, and killed by decapitation. The animals underwent thoracotomy and then proceeded to the isolation of thoracic aorta. After removal of the connective tissue, the aorta was divided into four rings. Two of the rings, selected randomly, had the endothelium removed mechanically. The rings were mounted in organ baths containing modified Krebs-Henseleit solution (composition in mM: NaCl 113.0; KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO 4 1.1, NaHCO3 25.0; glucose 11.0, ascorbic acid 0.11). The nutrient solution was maintained at 37.0 ± 0.5°C and saturated with carbogen (95% O2, 5% CO2). The rings were connected to isometric force transducers coupled to a physiograph (Ugo Basile). The settling time was 1 h under an initial tension of 1.5 g maintained throughout the experiment. A resting tension of 1.5 g is optimal in producing the maximum contraction to noradrenaline (NA) in the aorta of adult rats (Cordellini et al., 2006). The rings of thoracic aorta with endothelium (+E) and without (-E) were studied in parallel (Cordellini et al., 1990, 2006). Cumulative concentration-effect curves to NA were constructed from the tissue responses to NA. The increased doses produced remained stable over approximately 40 to 60 s (Cordellini et al., 1990, 2006).

The responses to NA were also examined in the absence and presence of L-NAME (3 × 10^4 M), an inhibitor of nitric oxide synthase, which was added to the preparations in the last 30 min of stabilization and remained in contact with them until the end of the experiments. Following completion of the determination of the concentration-effect curves, single doses of acetylcholine (10^4 M) and sodium nitroprusside (10^-4 M) were used to test the integrity of the endothelial cell and smooth muscle layer, respectively. Vascular reactivity was assessed by the change of the maximum response (g voltage) and power (50% effective concentration - EC50) of the NA in individual experiments. The EC50 is defined as the molar concentration of agonist needed to achieve an effect equal to 50% of maximal effect.

Wet weight of the organs and microscopic examination of the liver

The animals were weighed every 2 days until the end of treatment. After euthanasia, the internal organs were removed for macroscopic verification. Immediately after the sacrifice, the organs were weighed. The livers were stored for histological evaluation and sectioned into three portions to obtain tissue samples. The samples were kept in 10% formalin solution for 24 h, washed in tap water, and stored in 70% alcohol until paraffin embedding; 20-mm thick sections were made, and the slices were stained with hematoxylin/eosin.

Statistical analysis

Data were compared by one way analysis of variance (ANOVA) followed by the Tukey’s post-test for the presence of significance (Sigma Stat 3.5). For behavioural data, non-parametric Mann-Whitney test was used. Results were expressed as the mean ± standard error of mean (SEM). The significance level was 5%.

RESULTS

Phytochemical evaluation

The qualitative phytochemical analysis of the S. erecta root detected the presence of saponisins (foaming test),
flavonoids (magnesium chip and hydrochloric acid test), tannins (reaction of ferric chloride and 2% gelatin in drip test), catechins (brown-yellow colour after acid-base reaction) and cardiac glycosides (Kedde reaction).

Administration of decoction

The animals' body weight was similar in the two groups throughout the experiment. The blood glucose levels analysed between groups were treated SHR: 97.0 ± 0.5 vs. control SHR: 96.5 ± 0.5 mg/dl; treated WKY: 96.6 ± 0.6 vs. control WKY 96.7 ± 0.6 mg/dl.

Exploratory and anxiety tests: OF and EPM

In the OF, we were interested in studying the exploratory behaviour; a decrease in this behaviour by the decoction would be considered a signal of central effects, for instance, a sedative action. There were no differences between the experimental groups (p > 0.05) (Table 1). The EPM considers the time spent in each part of the apparatus, that is, the closed or open arms, in order to infer the level of anxiety. There were behavioural differences between strains in the time spent in the open arms (p = 0.01), and the treatment did not alter this difference (Table 2).

Blood pressure and heart rate

Table 3 presents the data obtained for treated and untreated SHR and WKY. There were differences between strains in MAP, evidencing hypertension for SHR (p < 0.01). The treatment was not able to change this difference. The heart rate was normal in both groups and was not altered by the treatment (p > 0.05).

Vascular reactivity tests

Regardless of endothelium integrity, the reactivity to NA in rings of thoracic aorta was similar in SHR and WKY. Treatment with S. erecta in WKY rats did not cause any change in reactivity to NA for either (+E) or (-E)
Table 4. Concentration-effect to noradrenaline in the absence or presence of L-NAME obtained in the thoracic aorta ring with or without endothelium of the WKY and SHR rats treated or not.

<table>
<thead>
<tr>
<th>Aortic rings condition</th>
<th>Treated SHR (n = 7)</th>
<th>Control SHR (n = 8)</th>
<th>Treated WKY (n = 5)</th>
<th>Control WKY (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA (+E)</td>
<td>0.92 ± 0.18*</td>
<td>1.68 ± 0.26</td>
<td>1.78 ± 0.37</td>
<td>1.83 ± 0.49</td>
</tr>
<tr>
<td>L-NAME (+E)</td>
<td>1.94 ± 0.30</td>
<td>1.60 ± 0.13</td>
<td>2.03 ± 0.37</td>
<td>2.44 ± 0.11</td>
</tr>
<tr>
<td>NA (-E)</td>
<td>2.65 ± 0.45</td>
<td>2.10 ± 0.20</td>
<td>2.35 ± 0.30</td>
<td>3.08 ± 0.28</td>
</tr>
<tr>
<td>L-NAME (-E)</td>
<td>1.88 ± 0.17</td>
<td>1.77 ± 0.21</td>
<td>2.28 ± 0.15</td>
<td>2.73 ± 0.21</td>
</tr>
</tbody>
</table>

NA: Noradrenaline; (+E): with endothelium; (-E): without endothelium. Data is given in grams (g) of contraction. Results are expressed as mean ± standard error of the mean. One way analysis of variance (ANOVA) followed by the Tukey's post test showed differences for (+E) groups.*different from control SHR and WKY strain (p = 0.03), different from NA (+E)/treated SHR (p = 0.02).

Table 5. Wet weight of the organs and body weight at the end of the treatment.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Treated SHR (n = 9)</th>
<th>Control SHR (n = 10)</th>
<th>Treated WKY (n = 7)</th>
<th>Control WKY (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.409 ± 0.019</td>
<td>0.407 ± 0.007</td>
<td>0.310 ± 0.013</td>
<td>0.300 ± 0.011</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.521 ± 0.017</td>
<td>0.483 ± 0.032</td>
<td>0.461 ± 0.021</td>
<td>0.450 ± 0.031</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.515 ± 0.018</td>
<td>0.506 ± 0.015</td>
<td>0.490 ± 0.029</td>
<td>0.548 ± 0.037</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.189 ± 0.006</td>
<td>0.209 ± 0.008</td>
<td>0.258 ± 0.005</td>
<td>0.239 ± 0.002</td>
</tr>
<tr>
<td>Liver</td>
<td>4.193 ± 0.162</td>
<td>4.150 ± 0.136</td>
<td>4.146 ± 0.139</td>
<td>4.085 ± 0.190</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.409 ± 0.019</td>
<td>0.409 ± 0.019</td>
<td>0.789 ± 0.024</td>
<td>0.831 ± 0.027</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.023 ± 0.002</td>
<td>0.025 ± 0.002</td>
<td>0.016 ± 0.003</td>
<td>0.022 ± 0.002</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>334.4 ± 6.1</td>
<td>327.9 ± 4.3</td>
<td>405.3 ± 10.1</td>
<td>431.2 ± 12.8</td>
</tr>
</tbody>
</table>

The organ weight was corrected for each 100 g of body weight. Results are expressed as mean ± standard error of the mean. One way analysis of variance (ANOVA) showed no difference between the weights of organs for each SHR or WKY, treated or control, p > 0.05.

(Table 4). However, after treatment, there was a decrease in the maximum response to NA in aorta (+E) in SHR (treated SHR: 0.92 ± 0.18 g vs. control SHR: 1.68 ± 0.26 g, p = 0.02), which was abolished by the presence of L-NAME, an inhibitor of nitric oxide synthase (treated SHR: 1.94 ± 0.30 g, p = 0.03).

Wet weight of organs and microscopic examination of the liver

The wet weight of the organs was similar in both strains for both treated and control animals (Table 5). Macroscopically, there were no changes in internal organs after chronic administration of the decoction. Histological sections of the liver tissue of the animals showed no microscopic changes, such as fibrosis or pre-neoplastic lesions, after 32 days of treatment with the decoction.

DISCUSSION

The present results show that treatment with a decoction of S. erecta roots did not reduce blood pressure in SHR or WKY. However, chronic treatment was able to reduce the vascular reactivity to NA in treated SHR. In addition, this reduction may have been mediated by the activation of endothelial nitric oxide (NO). NO participation was indicated by the fact that L-NAME, a NO synthase inhibitor, reversed the reactivity to NA in the rings of the thoracic aorta (+E) in treated SHR. Treatment with the decoction did not change any anxiety parameters. In addition, chronic treatment with the decoction of S. erecta roots produced no macroscopic changes in the organs studied in either strain. The treatment did not alter the microscopic tissue structure of the liver, the primary metabolic organ of the body, which might indicate that this part of the plant is not toxic under these conditions.

The hypertension in SHR is attributed to a polygenic effect due to either neural or vascular disorders (Lerman et al., 2005). SHR is an interesting model in which to study possible effects of decreasing blood pressure levels because many features of hypertension in this strain are similar to those found in most cases of systemic hypertension in humans, that is, essential hypertension. For instance, this model is associated with cardiac hypertrophy, endothelial dysfunction (with increased vascular reactivity) and impaired renal function (Lerman et al., 2005; Li and Bukoski, 1993; Li et al.,...
An imbalance in the production or inactivation of NO contributes to the development and persistence of high blood pressure as well as the development of complications of hypertension (Higashi et al., 2002; Naseem, 2005; Panza et al., 1995). Thus, even though the MAP of treated SHR in this study were not reduced, the decoction of \textit{S. erecta} reduced vascular reactivity. The present study shows that in treated SHR, the endothelial function is restored, as the maximum response to NA is reduced in thoracic aorta rings (+E) in treated SHR without L-NAME as compared to control SHR.

The presence of L-NAME in treated SHR increased reactivity to NA in aorta rings (+E), compared to the same condition in the absence of L-NAME. No change in aortic response to NA was observed between SHR and WKY (-E). In this context, the observed alterations in the endothelial pathway of the aorta from treated SHR, but not treated WKY, were not surprising. There have been described, among other features, an increased synthesis of vasoconstrictor cyclooxygenase products and superoxide anions in vascular tissue of SHR (Garcia-Redondo et al., 2009; Gao and Lee, 2001). In order to counterbalance these vasoconstrictor factors, an increase in NO synthesis could be observed in the vascular tissue of SHR compared to WKY (Puzserova et al., 2014). In this context, \textit{S. erecta} treatment may reduce the SHR-increased vasoconstrictor factor production and thus unveil the NO pathway hyperactivation in SHR. NO is produced within the endothelium and then activated by intracellular mechanisms underneath vascular smooth muscle cells (Archer et al., 1994). The decoction of \textit{S. erecta} roots must contain a factor that favours these mechanisms and consequently increases NO supply to the smooth muscle of blood vessels, contributing to the decrease in vascular reactivity.

The role of NO is attributed to regulation of the mechanical properties of the arteries, in particular the great arteries, promoting aortic compliance (Joannides et al., 1997). The increase in compliance of the aorta favours the distribution of blood microcirculation (Joannides et al., 1997). Thus, even though the MAP was not reduced in this study, greater endothelial function in the aortic rings can translate into improved blood flow to the microcirculation, which is very beneficial for long-term tissue perfusion. It is worth noting that we used a relatively small dosage of the substance, 0.5 g of crude root administered only once a day. The administration of the decoction at a higher frequency than was used in the present study could decrease the mean arterial pressure. Normally, teas and decoctions are taken in 3 doses daily. In the future, other studies may better delineate this issue.

The Sapindaceae family is a rich source of secondary metabolites. Studies by Schmitt and Dirsch (2009) described the roles of various plant secondary metabolites favouring the production of NO in endothelial and cooperating endothelium-dependent vasodilation. This action occurs predominantly because of flavonoids, such as those found in \textit{S. erecta} (Chan et al., 2000). Phytochemical analysis of \textit{S. erecta} roots detected the presence of saponins, flavonoids, tannins, catechins and cardiac glycosides. Recent studies of the ethanol extract of the roots of \textit{S. erecta} showed the presence of flavonoid glycosides derived from kaempferol: kaempferol-3-O-α-L-rhamnopyranoside and kaempferol-3-O-α-L-rhamnopyranoside-(1 to 6)-β-D-glucopyranoside (Cardoso et al., 2013). In an aortic ring assay used to check the forward vascular reactivity to 5 different bioactive compounds, Leeya et al. (2010) showed that kaempferol has important vasoconstrictor activity via activation of NO. Moreover, Gasparotto et al. (2011) found that intravenous injections of kaempferol in anesthetized SHR were not able to produce a hypotensive effect. These results are similar to the present results, where chronic treatment with \textit{S. erecta} exerted a vasoconstrictor effect in aortic rings but produced no reduction in blood pressure. Because the findings of this study result from the administration of a decoction and not an isolated extract, we cannot say which substance is responsible for the positive effect on endothelial function, or whether there is a synergistic effect of various substances found in the decoction, but kaempferol seems to be a candidate for these actions. It is worth highlighting that there is an association between enhanced anxiety and high blood pressure values (Ginty et al., 2013; Garcez et al., 2014). Because greater interactivity and a certain docility of SHR treated with the decoction were observed during the treatment, we speculated that the decoction might have an anxiolytic or sedative effect. Using a combination of the OF and EPM tests, these behaviours may be inferred.

The decoction did not cause any differences in exploratory activity; the rearing and the number of squares crossed were similar between strains and treatment. The data for EPM were also similar within each strain. The SHR did not fear spending time in the open part of the apparatus, and the treatment did not change this. However, the WKY (treated or not) spent more time in the closed arms. The SHR strain has symptoms that are similar to those of attention deficit disorder, that is, hyperactivity and impulsivity, compared to WKY (Sagvolden, 2000; Sagvolden et al., 2005). Such symptoms may explain the apparent greater interactivity and docility of animals during treatment. On the other hand, WKY exhibits behavioural instability. Thus, the decoction of \textit{S. erecta} root in these conditions did not cause changes in exploratory activity parameters or anxiety. The chronic administration of the decoction did not produce any macroscopic changes in the internal organs or even any microscopic changes in the liver. Although toxicity tests are far more complex, involving measurements of the serum concentrations of some...
enzymes, our results may suggest that this part of the plant has no apparent toxic effects on organ structure. These findings are a promising sign that this treatment does not have serious side effects.

Conclusion

The present results showed a reduction in vascular reactivity produced by the S. erecta root decoction in SHR rats, indicating that this part of the plant might promote blood flow through microcirculation. Thus, conventional wisdom seems to be accurate with regard to the use of this part of the plant against hypertension, although further study is needed to identify the active ingredients responsible for this activity.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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